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14. ABSTRACT

The overarching objective of this study is to characterize abnormalities of vestibulo-ocular reflexes (VOR) in Autism Spectrum Disorder (ASD). Specific Aim 1: Characterize horizontal VOR post-rotary nystagmus without optokinetic feedback using a velocity step test. We hypothesize that in ASD vertical eye movement intrusions during horizontal nystagmus will occur more frequently than normal, will be time-locked to horizontal nystagmus, and will differ from voluntary saccades. Specific Aim 2: Characterize horizontal VOR without optokinetic feedback using sinusoidal oscillation tests. We hypothesize that gain and phase lag of horizontal VOR will differ in children with ASD compared to controls. Specific Aim 3: Characterize in ASD vertical VOR and torsional VOR, both without optokinetic feedback. The present report covers the first year following award initiation on 15 May 2010. Limited research data have been obtained due to equipment issues that have recently been solved.

15. SUBJECT TERMS

Vestibulo-ocular reflex, autism

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Table of Contents

INTRODUCTION

The overarching objective of this study is to characterize abnormalities of vestibulo-ocular reflexes (VOR) in Autism Spectrum Disorder (ASD). Specific Aim 1: Characterize horizontal VOR post-rotary nystagmus without optokinetic feedback using a velocity step test. We hypothesize that in ASD vertical eye movement intrusions during horizontal nystagmus will occur more frequently than normal, will be time-locked to horizontal nystagmus, and will differ from voluntary saccades. Specific Aim 2: Characterize horizontal VOR without optokinetic feedback using sinusoidal oscillation tests. We hypothesize that gain and phase lag of horizontal VOR will differ in children with ASD compared to controls. Specific Aim 3: Characterize in ASD vertical VOR and torsional VOR, both without optokinetic feedback. Because neither of these aspects of the VOR have been described previously in ASD, Aim 3 is exploratory.

The present report covers the first year following award initiation on 15 May 2010. Limited research data have been obtained during Year 1 due to equipment issues that were not solved until Year 1 Month 12.

BODY

Statement of Work: Abnormal Vestibulo-Ocular Reflexes in Autism: A Potential Endophenotype

Task 1. Activities preparatory to research (year 1, months 1 - 6)

Subtask 1a. Submit protocol for human research participation to UF Institutional Review Board.

Milestone #1: Human research participation approval by UF Institutional Review Board was granted in April 2010, renewed in April 2011, and granted by ARO Human Research Protections Office (HRPO Log No. A-16019). Minor changes to the protocol, such as creation of a phone screening form for recruitment, have been approved by the UF IRB and HRPO on several occasions. Milestone #1 is on track.

Subtask 1b. Research assistants trained in administration of Autism Diagnostic Observation Schedule (ADOS), certification required, and other testing administration.

Milestone #2: Neuropsychological and vestibulo-ocular reflex tests ready to be administered by research assistants. Neuropsychological testing kits and materials have been acquired. Tana Bleser, Graduate Research Assistant, has been certified for clinical and research administration of ADOS tests, and has also attended a special workshop for training on pediatric vestibular testing. Jill Weish, Graduate Research Assistant who joined the project at the start of Year 2, will complete her ADOS training July 2011. Milestone #2 is on track.

Subtask 1c. Submit protocol for recruitment of research participants to Alachua County School District, to local therapy centers having ASD clients, and to UF Center for Autism and Related Disorders (CARD).

Milestone #3: Permission granted to recruit on premises (schools, therapy centers) or via a contacts database (CARD). Milestone #3 is presently on track. Approximately 2000 recruitment fliers were distributed in public and private schools before the school year ended, and several therapy centers also have fliers. Participants are currently responding to those fliers.

Task 2. Acquire eye tracking apparatus and set it up on site integrated with existing equipment (year 1, months 1 - 9). Except that existing equipment had to be replaced, as described below, Task 2 was actually completed by Year 1 Month 12.

Subtask 2a. Establish appropriate levels of infrared illumination for high frame rate eye tracking while maintaining low visibility to the participants. This has been achieved.

Subtask 2b. Synchronize eye tracking data acquisition and rotary device motion control computers. This has been achieved.

Subtask 2c. Create data base structures to link eye tracking data and rotary motion data to neuro-psychological results. This work is currently in progress, it was not possible to start this subtask meaningfully until Year 1 Month 12.

Subtask 2d. (year 1, months 1 - 9) Research assistants trained in administration of vestibulo-ocular reflex tests, data entry procedures, and data quality control/quality assurance. This work is currently in progress, it was not possible to start this subtask meaningfully until Year 1 Month 12.

Milestone #4: Equipment and software ready for testing human participants. This milestone met Year 1 Month 12, a delay of 3 to 6 months caused by equipment issues.

Difficulty was experienced such that Task 2 and Milestone #4 were delayed. Proposed Task 2 was predicated upon the use of existing equipment to serve as the rotary platform to supply vestibular stimulation. During the first quarter (year 1, months 1 – 3) intense work on this existing equipment was carried out by the mechanical engineer who originally designed the existing rotary platform, a skilled undergraduate to assist him, and two advanced undergraduates in Electrical and Computer

Engineering. All four of these individuals had been working with the existing equipment prior to the start of the present project. During software testing to insure that the rotary platform could execute the motions required for the experimental design, a crack developed in the housing of the motor which moves the platform.   Advice from the engineers was that this existing equipment would not suffice.

We contacted manufacturers of clinical vestibular testing equipment to find out whether we could obtain replacement equipment. The manufacturer Neuro Kinetics Inc. was willing to consult with us to design suitable equipment to meet our specifications. With approval the ARO Contract Officer this equipment was purchased. It was installed in March 2011 but one of two computers used for controlling the equipment was defective. This computer was repaired by Dell in April 2011. By 14 May 2011, the end of the first project year, the equipment had been used successfully to collect preliminary data from ten college student volunteers, as approved by UF IRB and reported to HRPO.

The purpose of this preliminary data collection was to insure that results obtained with this equipment were representative, that is, in good correspondence with norms. Norms are more readily available for young adults than for children. Analyses of these preliminary data allowed us to detect and correct a small calibration error (an incorrect value in a control file).

Task 3. Recruitment and testing of 8 pilot study participants (year 1, months 6 - 12). Recruitment of research participants is from Alachua County public schools, local therapy centers having ASD clients, and the UF Center for Autism and Related Disorders (CARD). All research testing and laboratory work takes place at the University of Florida. Task 3 commenced Year 1 Month 12, having been delayed by equipment issues explained above.

Subtask 3a. (year 1, months 6 - 12) Recruitment of 4 ASD research participants from Alachua County public schools, local therapy centers, and UF Center for Autism and Related Disorders (CARD). Administration of the following questionnaires to each set of parent(s)/guardian(s): Children's Communication Checklist-2, Repetitive Behavior Scale-Revised, Vineland-II, and the ShortSensory Profile. Questionaire responses are scored and entered into database. Administration of neuropsychological tests to each ASD child: Autism Diagnostic Observation Schedule (ADOS) and Leiter test of non-verbal problem solving. Scoring and validation of neurpsychological tests and entry into database. Administration of vestibulo-ocular reflex tests to each ASD child. Individual-level analysis made of eye movements to insure valid data capture of the reflexes (data quality control). Individual-level audits made of database record integrity (quality assurance). This subtask is currently in progress. . There have been minor changes to the specifc forms of neuro- psychological tests to be administered, as approved by UF IRB and HRPO.

Subtask 3b. (year 1, months 6 - 12) Recruitment of 4 non-ASD control participants from Alachua County public schools. This subtask will not begin until 10 ASD participants (50% of the target ASD sample size in Subtask 4a) have been recruited, to allow for the selection of controls who are (as group averages) age-and gender-matched to the ASD participants. Administration of the following questionnaires to parents: Children's Communication Checklist-2, Repetitive Behavior Scale-Revised, Vineland-II, and the Short Sensory Profile. Questionaire responses are scored and entered into database. Administration of Leiter test of non-verbal problem solving to each non-ASD (control) child, which is scored and scores entered into the database. Administration of vestibulo-ocular reflex tests to each non-ASD age- and gender-matched control child. Individual-level analysis of eye movements made to insure valid data capture of the reflexes (data quality control). Individual-level audits made of database record integrity (quality assurance). This subtask is currently in progress. There have been minor changes to the specifc forms of neuro- psychological tests to be administered to child participants, as approved by UF IRB and HRPO.

Subtask 3c. (year 1, month 12) Compare results from 4 non-ASD pilot participants to the literature for comparability. This subtask cannot be carried out at the present time. I believe it will be delayed until roughly Year 2 Month 3 to Month 6.

Subtask 3d. (year 1, month 12) Compare results from 4 ASD pilot participants to preliminary findings cited in the proposal for consistency. This subtask cannot be carried out at the present time. I believe it will be delayed until roughly Year 2 Month 3 to Month 6.

Subtask 3e. (year 1, month 12) Correct inefficiencies, if found, in test administration procedures or software or data structures. This subtask cannot be carried out at the present time. I believe it will be delayed until roughly Year 2 Month 3 to Month 6.

Subtask 3f. (year 1, month 12) Prepare abstract of pilot study findings for presentation at a national professional meeting. This will include preliminary analyses of results collected to date. This subtask cannot be carried out at the present time. I believe it will be delayed until roughly Year 2 Month 3 to Month 6.

 Milestone #5: Pilot study of 8 participants supports launch of formal research protocol by the end of grant year 1. Milestone #5 has not been met due to delay caused by equipment issues. I believe Milestone #5 will be met roughly Year 2 Month 3 to Month 6.

Task 4. Prepare annual report of grant activities with pilot study results and tentative conclusions from these pilot results (year 1, month 12). Accomplished June 2011.

Subtask 4a. (year 1, months 11 - 12) Annual renewal of human research participation approvals (UF IRB and HRPO). Accomplished April 2011.

Summary:

Activities preparatory to research (Task 1 and Task 2, Milestones #1 - #4) have been completed, except for ADOS training of the Graduate Research Assistant who joined the project at the start of Year 2. Her ADOS training takes place in July 2011.

Task 3 and Milestone #5 are currently in progress. Potential child participants are currently responding to recruitment fliers to be scheduled for testing. Delay in meeting Milestone #4 has in turn delayed meeting Milestone #5. Task 4 has been accomplished.

By 14 May 2011, the end of the award year 1, the new equipment had been used successfully to collect data from ten college student volunteers, as approved by UF IRB and reported to HRPO.   The purpose of collecting these data was to insure that individual-level VOR results obtained with the new equipment were in good correspondence with norms, as a form of data quality control. The results obtained were satisfactory. These ten young adults are the only participants recruited and tested (VOR only) during grant year 1.

KEY RESEARCH ACCOMPLISHMENTS

• Equipment needed to study vestibulo-ocular reflexes, and to compare these reflexes between children with and without Autism Spectrum Disorder, is ready to test research participants.

• Ten young adult volunteer participants were tested with this equipment, and the data obtained were of good quality and within norms.

REPORTABLE OUTCOMES

- One manuscript submitted for journal publication. The manuscript is given in the Appendix.

Please note: Results reported in this manuscript were obtained prior to the grant award. The manuscript was written and submitted while the grant award was in effect.

- Bleser, T. & White, K. (2011) Abnormal Vestibulo-Ocular Reflex in Autism. Presented at the Midwinter Meeting of the Association for Research in Ototlaryngology, Feb. 19-23, 2011, Baltimore MD.

Abstract:

Objectives: To develop methods for studying vestibulo-ocular reflexes (VOR) in children with autism spectrum disorders (ASD) for replicating and extending previous findings of abnormal VOR in ASD.

Background: Oculomotor and postural control deficits are commonly noted in children with ASD, yet the neurological underpinnings of these deficits are poorly understood. Typical and atypical VORs have been well studied and provide an excellent resource for studying neural mechanisms of visual-vestibular deficits in ASD.

Methods: Our lab developed a pediatric rotary chair (P-ROC) for testing VOR in children with ASD. Three children with ASD and three age-matched normal controls 6-12 years old participated in velocity step tests. Videonystagmography (VNG) recordings were taken during and after rotation for 8 trials. ASD diagnoses were confirmed by the Autism Diagnostic Observation Schedule (Lord et al., 2000).

Results: Although our sample is small and our sampling rate low (30 fps), our results are robust and replicated those of Ritvo et al. (1969). The ASD group compared to controls showed (a) significantly decreased duration of post-rotary nystagmus in the light (ASD = 6.3 sec and controls = 14.4 sec, $p<0.001$) and (b) no difference in the duration of post-rotary nystagmus in the dark (ASD = 31.6 sec and control group = 30.2 sec, $p=0.091$). Unlike Ritvo et al. (1969), we used VNG rather than ENG, which enabled us to identify additional aberrations in the quality of VOR in ASD which were not previously reported. We are currently analyzing these data and expect significant differences between the two groups.

Implications: VORs can be objectively measured early in development, thus, if certain deficits are specific to ASDs, such deficits may help identify risk early. VORs can also be used as treatment outcome measures or used for patient-treatment matching in ASD. Future studies will aim to thoroughly characterize abnormalities and elucidate the underlying neural mechanisms.

Please note: Results reported in this abstract were obtained prior to the grant award. The abstract was prepared and delivered while the grant award was in effect.

- Bleser, T. & White, K. (2011) Visual and Vestibular Integration Deficits in Autism: New Findings. Presented at the American Occupational Therapy Assn. 91st Annual Conference & Exhibition, April 14–17, 2011, Philadelphia PA

Abstract:

Pilot study results of abnormalities in the vestibulo-ocular reflex of children with ASD will be presented. The implications of these findings to the field of occupational therapy and sensory integration treatments for children with autism will be discussed.

Please note: Results reported in this abstract were obtained prior to the grant award. The abstract was prepared and delivered while the grant award was in effect.

- Master of Science in Psychology awarded to Tana Marie Bleser on December 21, 2010. Title: "ABNORMAL HORIZONTAL VESTIBULO-OCULAR REFLEX IN AUTISM SPECTRUM DISORDERS."

Please note: Results reported in this thesis were obtained prior to the grant award. The thesis was written while the grant award was in effect.

CONCLUSION

Activities preparatory to research (Task 1 and Task 2) are on track. Task 2 relating to configuring the equipment was delayed significantly by equipment failure and the acquisition and installation of new equipment. The new equipment is functional as of Year 1 Month 12. This has delayed the ability to commence Task 3 by roughly six months. Task 3 is in progress.

REFERENCES

None

APPENDIX A

Title:

Abnormal Horizontal Vestibulo-Ocular Reflex in Autism Spectrum Disorders

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Abstract

Studies of vestibulo-ocular reflexes (VOR) in autism spectrum disorders (ASD) can address the two following concerns: (1) identification of early bio behavioral markers and (2) elucidation of the aberrant sensorimotor neuropathology. The current case-series of 3 children with ASD and 3 controls (age 6 – 10) tests a novel pediatric rotary platform and systematically replicates and extends previous findings of abnormal VOR in ASD (Ritvo et al., 1969). Velocity step testing was conducted in two conditions (i.e., light and dark) and both the duration and quality of post-rotary nystagmus (PRN) was measured with frame-by-frame video analysis. In the light, children with ASD show significantly decreased mean duration of PRN (6.2 sec) compared to controls (14.4 sec; Mann Whitney-U $p < 0.001$). In the dark, there is no significant difference between groups (Mann Whitney-U, $p = 0.091$), however, the ASD group exhibited increased frequency of vertically directed eye movements (Mann Whitney-U, $p = 0.001$). The quality of nystagmus is sufficiently different in ASD that novice observers can differentiate normal from abnormal videos. Future studies are warranted to characterize VOR in a larger ASD sample, to establish whether such aberrations occur early in development and to evaluate their sensitivity and specificity to ASD.

Key words: vestibular, oculomotor, autism, early diagnosis, vestibulo-ocular reflex

1.   Introduction

Autism spectrum disorders (ASD) are currently diagnosed on the basis of abnormal behavior within

three core domains: (1) social skills, (2) communication skills, and (3) restrictive/repetitive behaviors

[12]. Currently ASD are estimated to affect 1 in 110 children born in the US [8]. Early identification and

early intervention have been shown to have a significant effect on the prognosis for young children with

ASD [11,49]. However, an early diagnosis of ASD is currently limited to children who are approximately 2

to 3 years of age [31,37,50]. Yet, it is clear that the abnormal neurobiological processes which result in

ASD occur during fetal development [47,48] and/or infancy [10] long before the onset of the classic

behavioral symptoms currently used to affirm a diagnosis. Thus, identification of a bio-behavioral

marker that occurs early in development and is related to the neurobiology of ASD would be particularly

useful both for earlier identification of risk and for understanding the neuropathological processes

resulting in ASD.

Beyond the three main behavioral domains of ASD, deficits in sensory processing and motor

coordination are also of concern and have been observed young children less than 2 to 3 years of age

who were later diagnosed with ASD [3,22,26,56,58,60]. Thus, sensory and motor abnormalities may

currently provide the earliest warning signs of risk for ASD.

The vestibulo-ocular reflex (VOR) is a promising candidate for an early sensorimotor bio-behavioral

marker of risk for ASD for several reasons. First, horizontal VOR has been previously shown to be

abnormal in young children with ASD [38,45]. Second, the VOR involves integration of sensory and

motor information at sites that have been shown to have morphological abnormalities in ASD such as the brainstem ([46] Rodier, 2002; [21] Jou et al., 2009), cerebellum [53], thalamus and parietal lobes (see [5] and [57] for reviews). Third, the anatomy and physiology of the VOR is one of the best studied of all the vestibular and postural reflexes and provides a solid foundation for studying this reflex and its related neurobiology in ASD. Fourth, the VOR can be measured reliably in infants as young as 6 months of age [40]; hence, if a VOR marker were selective for ASD, then this reflex may provide a promising means for early identification of ASD risk. Lastly, the VOR is highly modifiable [6,52] therefore, it is reasonable to suspect that at least some of the deficits of VOR in ASD could respond to vestibular rehabilitation interventions.

Ritvo and colleagues (1969) reported the earliest finding of abnormal VOR in children with ASD. Horizontal VOR was tested in two conditions: (1) in the light with surroundings visible to the participant and (2) in the dark with surroundings not visible to the participant (i.e., children were blindfolded). In the light, the children with ASD showed significantly decreased duration of post-rotary nystagmus compared to typically developing children, whereas in the dark, there was no difference in the duration of post-rotary nystagmus between the two groups [45]. The authors concluded that children with ASD may be able to use optokinetic feedback for inhibiting post-rotary nystagmus to a greater extent than can typically developing children and that aberrations in brainstem or cortical input may be responsible for this difference.

Subsequent studies have reported additional abnormal characteristics in the VOR of children with ASD such as increased time constants of decay during per-rotary nystagmus and increased occurrence of

slow phases that failed to be followed by a quick phase reset, yet, show normal gain and peak slow

phase velocity [38]. These results, combined with those reported by Ritvo et al. [45] indicate that

aberrations in VOR may arise in regions of the central nervous system that modulate VOR and

oculomotor control such as the brainstem nuclei, the cerebellum or the basal ganglia circuitry, rather

than in the peripheral vestibular organs.

One other study has further investigated VOR in ASD based on neuroanatomical evidence of decreased

Purkinje cells and/or decreased volume in cerebellum vermian lobules I-V [2,44], but not lobules VIII-X

[14,18,20] in high-functioning autism (HFA). Since lobules IX and X (the nodulus and uvula respectively)

are known to be involved in the maintenance of sustained VOR, Goldberg et al. [16] hypothesized that if

lobules IX and X are spared in HFA, then tilt suppression of VOR should be normal in this population.

They conducted a test of tilt suppression following velocity step test and found that children with HFA,

indeed, show no difference in tilt suppression of VOR compared to typically developing controls when

tested in the dark. The normal tilt suppression of post-rotary nystagmus in ASD is consistent with the

interpretation that, at least for individuals with HFA, vermian lobules IX and X of the cerebellum are

spared from the otherwise consistent neuroanatomical findings of morphological abnormalities in other

areas of the cerebellum in ASD [5]. However, in light of evidence that low-functioning ASD individuals do

show structural differences in lobules IX and X [19,42] it is possible that deficits in sustaining VOR may

exist in the lower-functioning ASD population. Therefore, questions remain about VOR abnormalities

and correlations between VOR and cerebellar abnormalities.

The first aim of the current study is to develop and test a novel apparatus for pediatric vestibular *en bloc*

rotational testing to improve tolerance of the methods used for studying VOR in children with ASD. In

particular, we hoped to have methods tolerated by the relatively understudied low-functioning ASD

population. The second aim of the current study is to conduct a pilot study to systematically replicate

and extend previous findings of abnormal VOR in children with ASD compared to typically developing

controls. The observations of interest for this study are the duration of post-rotary nystagmus and the

quality of per- and post-rotary nystagmus under two conditions: (1) in the light with surroundings visible

and (2) in the dark with surroundings not visible. We hypothesize that (a) in the light, children with ASD

will have significantly decreased duration of post-rotary nystagmus compared to typically developing

controls, (b) in the dark, there will be no significant difference in duration of post-rotary nystagmus

between the two groups, and (c) the quality of VOR eye movements in ASD will be significantly different

than that of controls.

2. Research Design and Methods

2.1.   Participants and Recruitment

Three typically developing children and three children with ASD between the ages of 6 and 10 years of

age participated in this study. All participants were recruited from local therapy centers and elementary

schools in Gainesville, Florida. The ASD group included three male children with diagnoses of ASD as

follows: Autism (8 years 6 months), PDD-NOS (7 years 3 months), and Asperger's Syndrome (10 years 6

months). The control group consisted of three typically developing children including two males (10

years 5 months and 7 years 4 months) and one female (6 years 9 months). For the ASD group, diagnoses within the Autism Spectrum were initially provided by parents/guardians upon recruitment and were confirmed in the laboratory by both the Autism Diagnostic Observation Schedule (ADOS) [32] and the Social Communication Questionnaire (SCQ) [51]. The ADOS is a play-based observation schedule that is currently considered the gold standard for identifying autism spectrum disorders when paired with the Autism Diagnostic Interviewer-Revised (ADI-R) [33]. The SCQ is a parent check-list used to screen for deficits in communication and social skills related to ASD. All three participants in the ASD group met criteria for diagnoses within the autism spectrum according to both their ADOS and the SCQ scores. Both assessments were administered by a neuropsychologist. All procedures were approved by the Institutional Review Board at the University of Florida. Children with ASD were provided an instructional video to watch at home with their families which detailed the activities and instructions for vestibular testing procedures.

2.1.   Pediatric Rotary Chair (P-ROC)

For this preliminary study, our lab developed a prototype of a pediatric vestibular rotary chair (P-ROC). The P-ROC prototype for the current study (Figures 1 – 3) includes a circular padded platform approximately 3 feet in diameter that rotates around a central axis and is powered by a motor and video cameras that record eye movements during and after rotation. The platform provides a surface for the child to sit while rotating around a central axis (similar to a miniature merry-go-round). A padded post rises up through the center of the platform providing the axis of rotation as well as a mainstay and postural support for the participant. The headrest and video cameras are also mounted on this center post. Below the platform, four support beams extend outward from the central axis to support the

platform and the weight of the participant. At the top of the padded post, the headrest is pitched

downward from vertical 30° to promote preferential stimulation of the horizontal semi-circular canals.

The headrest adjusts in the upward and downward direction for participants of different heights and the

forehead and chin cushions adjust inward and outward to fit any size face. Below the platform is a

planetary gear reduction winch with a 12 volt DC powered motor and a drum that has been modified to

accept a belt. This facilitates the ability to vary the ratio to the main shaft by selection of different sized

pulleys. The control circuit of the motor uses a series of resistors and relays to achieve the desired

steady speed in both directions. This motor is mounted to the base and rotates the center post and

platform. The motor controls the start and stop of clockwise or counter clockwise rotation. An infrared

(IR) video camera is mounted to the top of the center post and records eye movements through the hole

in the front of the headrest. For testing sessions in the dark condition, a cover including an IR filter

(Edmund Optics Optical Cast IR Long-pass Filter) is mounted to the outside of the headrest between the

child's face and the camera which blocks all visible light and allows only IR and near IR light to pass

through to the participant's face. Several additional filters were placed on the cameras IR source to

prevent any visible light from reaching the participant's eyes. These IR filters enable the video cameras

to view the eyes while preventing the participant from viewing his/her surroundings.

2.1.1.   Vestibulo-ocular Reflex Testing Procedure

Two investigators were present for all vestibular testing; one operated the device and monitored data

acquisition while the other monitored the participant's comfort and safety during testing. Each

participant was provided 5 to 10 minutes at the beginning of the testing session to acclimate to their

surroundings. During this time they were also able to observe a demonstration of the rotary testing

procedure on the P-ROC. Since all children in the current study used verbal communication, they were

able to provide their verbal assent to participate. Upon obtaining their assent, the investigators assisted

the child into an upright sitting position at the center of the P-ROC platform with his/her arms and legs

crisscrossed around the center post. The safety harness was worn like a vest and attached via straps to

the center post in order to secure the participant to the P-ROC (Figure 2.). For the en bloc testing

procedure each participant attempted 8 trials of rotation total including 4 trials in two conditions: (1) in

the light with surroundings visible and (2) in the dark with surroundings not visible. In each condition we

conducted 2 trials in the clockwise direction and 2 in the counterclockwise direction in a

counterbalanced design. Each trial consisted of rapid acceleration to a constant velocity of 180

degrees/second for 20 seconds, followed by a stationary period of 30-40 seconds. During rotation per-

rotary nystagmus was recorded and during the stationary period PRN was recorded. All video recordings

were monocular recordings of the right eye only. Between each trial, participants were given a resting

period of approximately 60 seconds after nystagmus ceased. Ritvo et al. [45] previously noted that 30 to

90 seconds of rest between trials is effective for preventing habituation to the vestibular stimulation.

The testing session took approximately 30 minutes total. Participants were given a 5 minute resting

period after testing to watch a short cartoon. During this time, the investigators monitored the child for

any dizziness before allowing them to walk out of the lab.

2.2. Data Analyses

2.4.1.   Post-Rotary Nystagmus Duration. The duration of PRN was analyzed with frame-by-frame

analysis methods by two trained observers. Any disagreements between the two observers larger than

0.5 seconds were resolved by a third observer. The duration of PRN was measured by calculating the

difference between the time point at which the device stopped rotating and the time point at which the

nystagmus beats ceased to occur. The IR camera used for this pilot study has a sampling rate of 30

frames per second. The cessation of nystagmus was indicated by a fixational pause lasting longer than

210 msec ($\geq$ 6 video frames).

2.4.2. Quality of Rotary Nystagmus. Upon initial analysis of the duration of PRN, the observers noted

peculiar qualities in the eye movements of the participants in the ASD group which made it possible to

distinguish ASD videos from control videos. Therefore, two post-hoc analyses were conducted as

follows:

- *The first post-hoc analysis* included frame-by-frame video analysis to investigate the possible

  difference in the frequency of vertically directed eye movements between the two groups. One

  video of each participant in the dark condition was observed by two trained observers. For each

  frame of video, the direction of eye movement was recorded as either horizontal or non-

  horizontal; hereto after referred to as "vertical" eye movements including up-ward, down-ward,

  or diagonal eye movements. This analysis was conducted only on videos taken in the dark

  condition in an effort to minimize the possibility that the vertical eye movements were

  voluntary attempts to look at objects in the environment.

- *The second post-hoc analysis* was conducted to test the hypothesis that the VOR of children with

  ASD is sufficiently abnormal that a novice observer can distinguish ASD from control videos.

  Seventeen novice undergraduate students 19 to 23 years of age volunteered to participate as

observers for this study. Observers had no prior knowledge of VOR or nystagmus and were provided a brief training session including a written definition of normal VOR, a sample video of normal nystagmus and a simple rubric for distinguishing normal and abnormal nystagmus (Table 1). The sample video of normal nystagmus featured a typically developing participant who was not included in the study because he is the twin of another control participant. Observers were blind to group assignment and were presented 2 videos from each of the six participants. The 12 videos were presented in random order to each observer. Each video included 20 seconds of per-rotary nystagmus followed by 20 seconds of PRN.

3.   Results

3.1.   *Duration of Post-Rotary Nystagmus*

The mean duration of PRN was calculated for each participant (Figure 4-5) and for each group (Table 2) in both conditions. In the light, the ASD group showed significantly decreased mean duration of post-rotatory nystagmus (6.2 sec) compared to controls (14.4 sec; Mann Whitney-U $p < 0.001$; Figure 4). In the dark, there was no significant difference between groups (ASD = 31.6 sec and control group = 30.2 sec, Mann Whitney-U, $p = 0.091$; Figure 5). Out of 48 possible trials total, 7 ASD and 4 control trials were thrown out due to artifacts including low video camera resolution, failure to follow instructions, error in testing procedure by investigators, visible head movements during testing, excessive blinking, or the participant's election to end the testing session.

3.1.1. Quality of Rotary Nsytagmus

Two post-hoc pilot analyses evaluated the abnormal quality of VOR in ASD. The results of the first analysis show that the frequency of vertical eye movements during PRN is significantly higher in children with ASD (Table 3) compared to controls (Mann Whitney-U, p = 0.001). There was no significant difference in the frequency of horizontal eye movements between groups (Mann Whitney-U, p = 0.350). The results of the second qualitative analysis of VOR show that on average, a novice observer can correctly label 4.88 $\pm$ 1.23 out of 6 control videos as "normal". Observers on average can also correctly identify 4.47 $\pm$ 1.09 out of 6 ASD videos as "abnormal" (Table 4). Out of 17 observers, 88% identified 4 or more control videos out of 6 correctly as "normal" and 71% identified 4 or more ASD videos out of 6 correctly as "abnormal". Observers showed satisfactory intra-rater agreement when they labeled both videos of a given participant identically regardless of whether the labels were correct or incorrect. On average, observers agreed with themselves on 2.06 $\pm$ 1.0 out of 3 control participants and 1.71 $\pm$ 0.68 out of 3 ASD participants (Table 5).

4.   Discussion

4.1.   Pediatric Rotary Chair (P-ROC)

Our lab designed the P-ROC to be more conducive to pediatric testing than conventional adult rotary chairs, particularly for testing children with ASD and related disabilities. One benefit to the P-ROC that is particularly important for children with ASD is that it allows the child to be tested independently (i.e.,

without the need for the child to sit on an adult's lap). Additionally, the safety vest and headrest provide

postural stability, which is often deficient in individuals with developmental disabilities. The safety vest

is very similar to some of the compression vests that children with autism might experience in a therapy

or school setting and was well tolerated in our sample of children. Furthermore, our novel method of

VNG recording of eye movements without goggles is less invasive than other methods of eye tracking for

pediatric vestibular assessments such as electronysagmography (ENG), videonystagmography (VNG)

with goggles, or scleral coil implants [40]. The rationale behind attempting to use VNG without goggles

was that it might help to increase our ability to test low-functioning children with autism who might not

tolerate goggles on their face. From a methodological standpoint, infrared VNG is advantageous because

it enables the observation of eye movements with trajectories other than horizontal and allows the

recording of amplitude that is typically lost in band-pass filters when using ENG. Additional advantages

to the P-ROC include the small portable design and the fact that it does not require any kind of

enclosure. Overall, the replication of earlier VOR findings in both children with ASD as well as typically

developing children provides support for further use of the P-ROC in pediatrics.

Future versions of this apparatus and methods will include modifications that will improve both data

collection and analysis. The addition of methods for measuring the position and angular acceleration of

the head via sensors will help to manage possible confounding variables related to head movements.

Future versions of the VNG systems used with this apparatus should have higher sampling rates and

should include eye-tracking software that is capable of measuring the degree of angular rotation,

velocity, acceleration, gain, phase and symmetry of nystagmus. Future versions of the platform will

include adaptations that enable off-vertical-axis rotational testing and testing of infants and toddlers.

4.1.   Duration of Post-Rotary Nystagmus

Our results agree with previous findings [45] that children with ASD compared to typically developing, age-matched controls show (a) significantly decreased duration of PRN in the light and (b) no difference in the duration of PRN in the dark. The effect of decreased duration of PRN in the light is quite robust and highly replicable, even in a very small sample of children. Previously, this effect was interpreted as possibly occurring due to an increased ability for children with ASD to focus on visual information and thus enhancing the normal optokinetic suppression of nystagmus. We propose an extension of the previous interpretation based on a recent theory presented by [4]. The phenomena of decreased VOR in the light may be related to deficits in central coherence in ASD [13]. For instance, individuals with ASD have a tendency to focus on the details or parts of an object, scene or idea rather than the whole or gestalt of it (i.e., they fail to see the forest for the trees). If this hyper-vigilance to detail is pervasive throughout the brain in ASD, as suggested by Baron-Cohen, it may be a global process. This global central coherence problem may help to explain how individuals with ASD, through hyper-vigilence to detail of visual stimuli, can maximize the effect of optokinetic feedback for inhibiting nystagmus. This could be tested by providing a specific stimulus in the visual field following cessation of rotation in the light and tracking the participant's gaze within the visual field, noting what features they focus on, for how long and at what level of detail (i.e., different aspects of the entire object or one small detail of the object). Additionally, it would be interesting to compare the degree to which individuals with ASD can inhibit PRN and the degree to which they perform poorly on visual tests of central coherence that have previously been used in ASD [17].

4.2.   Quality of Rotary Nystagmus

While analyzing the duration of PRN, observers noted additional aberrations in the quality of nystagmus including abnormal characteristics of the slow phase of nystagmus such as failure to reset to center or failure to reset altogether as well as extreme excursion during slow phase eye movements. Ornitz et al., [38] noted similar deficits in the slow-phase excursions of horizontal PRN and reported that the frequency of their occurrence was significantly greater in children with ASD. The most striking observation in the present study is that children with ASD seem to exhibit much more frequent vertically directed eye movements during what should be purely horizontal nystagmus when tested in the dark. Such frequent vertical eye movements are unexpected during horizontal PRN and seem highly abnormal. Previous studies [38,45] used ENG to measure VOR eye movements, a method which may have misclassified vertical movements as blink artifacts or noise. The use of VNG in the current study, however, may have enabled us to observe, serendipitously, the increased frequency of vertical eye movements during horizontal VOR in children with ASD that have not been previously reported. Although both groups were subjected the same testing methods and, deficits in postural control in the ASD group may have contributed to slight, unobserved posterior head movements in this group that could potentially result in vertical eye movements. Any videos that included visible head movements were thrown out. Since it is possible to make voluntary saccades during rotary nystagmus, voluntary vertical eye movements are indeed possible and may explain the small number of vertical eye movements observed in controls. However, it seems unlikely that this is the reason for such a significant increase in the ASD group. Future studies should use higher sampling rates and software that can help to discriminate voluntary vertical saccades from reflexive, slow phase vertical eye movements.

A second analysis was conducted to determine whether the qualitative differences in VOR are sufficiently apparent that a novice observer is able to distinguish children with ASD and controls accurately. The results of this preliminary study indicate that with minimal training, observers without any prior experience with VOR or nystagmus can correctly identify normal VOR (e.g., control videos) vs. abnormal VOR (e.g., ASD videos) more often than not. On average when labeling multiple videos of the same participant, novice observers are both more accurate (i.e., labeling a video of a control as normal and ASD as abnormal) and more consistent (i.e., providing the same label to both of a participant's videos) when labeling control videos than ASD videos. This difference in observer accuracy and consistency between experimental groups may be because the observers' brief training provided more exposure to definitions and examples of normal nystagmus than abnormal nystagmus and therefore, observers may have been more confident in labeling videos of normal VOR. The question therefore arises as to whether a sample video of abnormal VOR and a more explicit description of the aberrations observed in ASD would improve the novice observers' abilities to label an ASD participant's eye movements as abnormal more consistently. It would also be useful to know if their lack of experience and familiarity with observing nystagmus eye movements in general hindered their ability to label abnormal nystagmus. Since a novice observer can accurately distinguish a video of ASD and control VOR more often than not, it would be reasonable to predict that clinicians with a background vestibular and/or oculomotor testing would perform even better at this task. Although the sample size is small, the results from this post-hoc analysis provide support for the idea that the abnormalities in the VOR in children with ASD hold promise to distinguish them from controls in a clinical setting with visual observation.

4.3.   Implications for ASD

It is clear that there are abnormalities in the VOR of children with ASD, but as of yet, it is unclear what is responsible for these abnormalities and whether these deficits are selective for ASD. *Normal* characteristics of rotational VOR in children with ASD include the duration of PRN [45], the tilt-suppression of PRN [16] and the gain of per-rotary nystagmus in the dark [38], indicating that the peripheral vestibular organs are probably healthy, particularly the semicircular canals. To date, there have been no studies of utricle or saccule function in ASD. *Abnormal* characteristics of VOR, when tested in the dark, include increased vertical intrusions (present study), increased time constants of decay, abnormal variability in slow phase velocity and in nystagmus beat patterns [38] which may be related to brainstem, cerebellum or basal ganglia circuitry [34]. When tested in the light, individuals with ASD show decreased duration of PRN [45, present study] which may be related to hyper-vigilance for visual stimuli or to deficits in visual-vestibular integration.

Normal vestibular function can have a large impact on the proper development of motor skills such as postural stability and locomotion [40], both of which have been reported to be abnormal in ASD. Studies of dynamic posturography, a sensory-selective method for evaluating the functional integration of these visual, vestibular and proprioceptive systems, have shown that maintaining postural stability is significantly more difficult for children with ASD. Children with ASD show postural control deficits particularly under conditions that occlude vision, diminish proprioceptive cues, and force the individual to rely on vestibular sensation [35,36]. Postural sway response to visual motion can also differentiate individuals with autism and Asperger's syndrome [15]. Such behavioral evidence further suggests that

28

the vestibular related deficits in ASD likely exist in central vestibular processing; however, the specific

central pathology is currently unknown.

In the central nervous system, the brainstem, cerebellum and basal ganglia circuitry are important for

modulation of the oculomotor system and the visual-vestibular interactions. The most consistently

reported neuroanatomical abnormalities in ASD are found in the cerebellum and the inferior olivary

nucleus of the brainstem (see [5] Bauman & Kemper, 2005 for review). Many post-mortem studies have

reported decreased size of the cerebellum and number of Purkinje cells (PCs) in the cerebellum of

individuals with ASD ([44] Ritvo et al., 1986; [2] Bailey et al., 1998; [25] Kemper & Bauman, 2002; [43]

Purcell et al., 2001; [28] Lee et al., 2002; [39] Palmen et al., 2004). The medial cerebellum includes the

vestibulocerebellum and the vermis which mediate vestibular sensory input and motor output

associated with oculomotor and postural control. The volume of the cerebellar vermian lobules I-V

[2,44] and VI-VII are decreased [1,7,9,23,24,41], whereas the volume of lobules VIII-X are possibly only

decreased in sub-groups with low IQ [16,20,42]. However, some have shown a decrease in vermis

volume in ASD that is not predicted by IQ [53]. Decreased PCs may cause notable aberrations in the

VOR including hypo- or hyper-metric nystagmus, as demonstrated by Hg toxicity in the cerebella of

guinea pigs [59]. Although previous studies have shown that the VOR gain in ASD is normal [38], they

did not account for IQ or level of function. Subtle dysmetria in saccade accuracy has been shown in ASD,

which may be related to the cerebellar pathology observed in ASD [54]. Additional neuroanatomical

abnormalities consistently noted in ASD include age-related morphological changes in the inferior

olivary nucleus of the brainstem [2] [39] [5]. The inferior olive plays an important role in oculomotor

control and vestibular processing. Motor learning in the cerebellum has been proposed to occur at the

synapses between Purkinje cells and the climbing fibers that extend from the inferior olive into the

cerebellum [29]. Additionally, abnormal eye movements have also been correlated with abnormal activity in fronto-striatal circuitry in ASD [55], areas of the brain that have been linked to the restrictive and repetitive behavioral symptoms of ASD [27,30]. Thus, aberrations in the VOR may be linked to multiple neurobiological areas of interest for ASD including the cerebellar, brainstem and fronto-striatal systems and could potentially serve as a model for studying sensorimotor processing deficits in ASD.

4.4.   Future Studies

Future studies should aim to establish whether or not abnormalities in the VOR are sensitive and specific to ASD and if they appear early on in development. If present early and specific to ASD, the VOR could provide the earliest bio behavioral marker for the identification of risk for ASD. The VOR also shows potential to serve as a bio-behavioral marker for ASD and may be used to create a model of sensorimotor integration deficits at the brainstem or cerebellar level. Alternatively, if VOR abnormalities are not specific to all ASD, but are selective for certain sub-groups within the autism spectrum, VOR could provide a bio-behavioral marker for identifying these sub-groups and for studying the neuropathological differences between sub-groups. Regardless of the VOR's selectivity for ASD, knowing exactly how it is different or deficient can provide a method for patient treatment matching with evidence-based pediatric vestibular rehabilitation interventions. Additionally, further studies aimed at thoroughly characterizing how the VOR is deficient in ASD may help to inform the development of new evidence-based visuo-vestibular interventions for individuals with ASD. It would furthermore be beneficial to know how early these aberrations in VOR are present, thus if specific to ASD, testing for these aberrations in younger children would be warranted.

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Figure 1.   Figure 2.   Figure 3.

Figures 1-3. The P-ROC is a pediatric rotary chair that consists of a spinning platform, headrest, motor and camera (Figure 1). The participant sits on the platform with his/her arms and legs wrapped around the center post and resting his/her head on the headrest (Figure 2). The camera is mounted to the central axis and is able to capture a clear view of the participant's eyes through the hole in the headrest (Figure 3).

Table 1. Guidelines for observers to use when labeling videos during post-hoc analysis.	
VOR eye movements should:	VOR eye movements should NOT:
• Move back and forth, from side-to-side	• Move up or down
• Move horizontally in a straight line	• Move in a circular pattern

Table 2.   Comparison of the mean duration of post-rotary nystagmus (in seconds) in each testing condition (light and dark) per group in two different studies.

	Ritvo (1969)		Bleser (2010)	
	Autism	Control	Autism	Control
Light	6.8	15.1	6.2	14.4
Dark	27.2	30.4	31.6	30.2

Table 3. Frequency of horizontal and vertical eye movements recorded during one trial in the dark for each participant.

	HORIZONTAL		VERTICAL	
	ASD	Control	ASD	Control
Participant 1	112	138	25	4
Participant 2	106	170	37	7
Participant 3	98	88	18	1

Table 4. Average distribution of observer responses (i.e., video labels) by experimental group (mean $\pm$ SD). Each cell is out of six possible videos (2 videos per participant, 3 participants per group).

	Normal	Abnormal
Control	4.88 $\pm$ 1.23	1.12 $\pm$ 1.23
ASD	1.53 $\pm$ 1.09	4.47 $\pm$ 1.09

Table 5. Average number of intra-rater agreements and disagreements between labels provided to the two videos per participant in each group (mean $\pm$ SD).

	Agree	Disagree
Control	2.06 ± 1.00	0.76 ± 0.88
ASD	1.71 ± 0.68	1.18 ± 0.73

**Duration of Post-Rotatory Nystagmus in
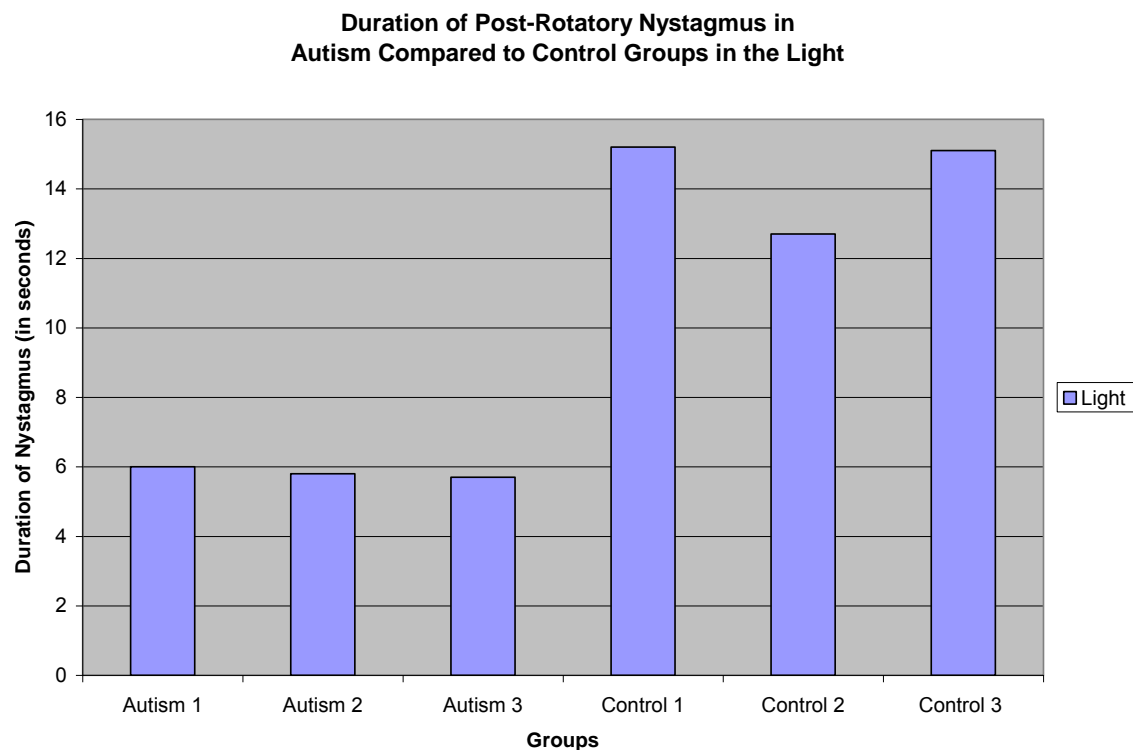Autism Compared to Control Groups in the Light**

Figure 4. Mean duration of post-rotary nystagmus (in seconds) for each participant for the light condition.

Participants with ASD showed significantly shorter duration of post-rotary nystagmus compared to control

participants (Mann-Whitney-U, $p < 0.001$).

**Duration of Post-Rotatory Nystagmus in
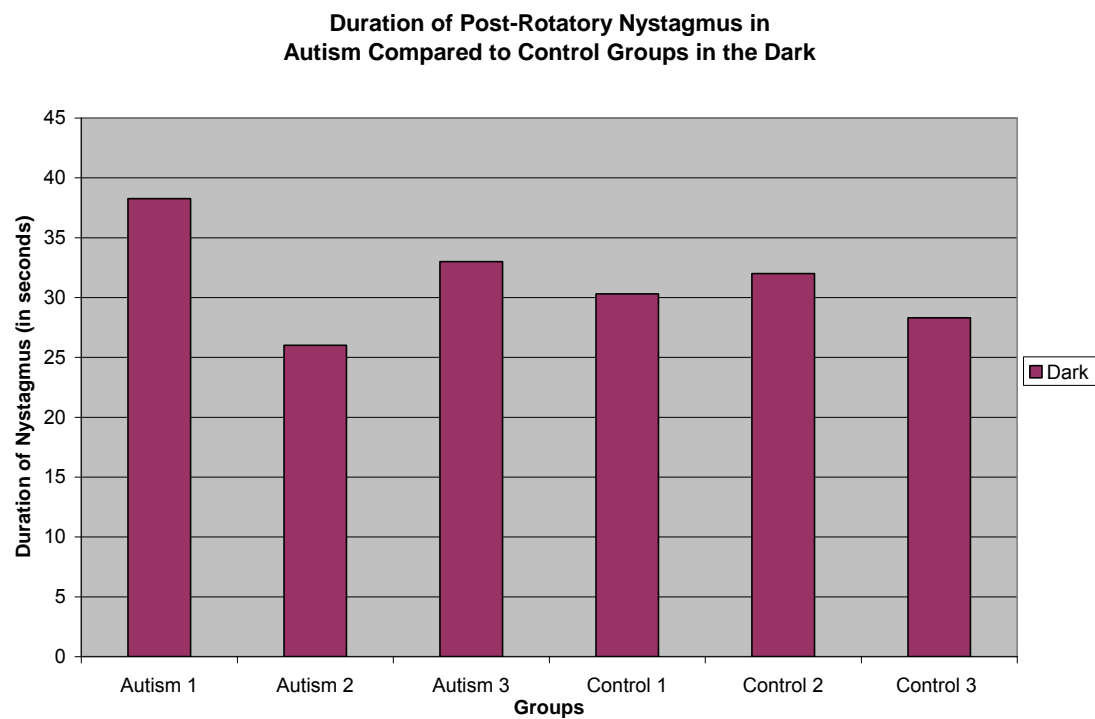Autism Compared to Control Groups in the Dark**

Figure 5. Mean duration of post-rotary nystagmus (in seconds) for each participant for the dark condition. There is no significant difference between groups (Mann-Whitney-U, p = 0.091).

SUPPORTING DATA

None